Application No. 10/542,217 Amendment dated February 14, 2008 Reply to Office Action of November 14, 2007

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Docket No.: SAE-0036

APPENDIX B

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A more recent version of this article appeared on July 3, 2003

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Identification of p2y9/GPR23 as a novel G protein-coupled receptor for Lysophosphatidic acid, structurally distant from the Edg family

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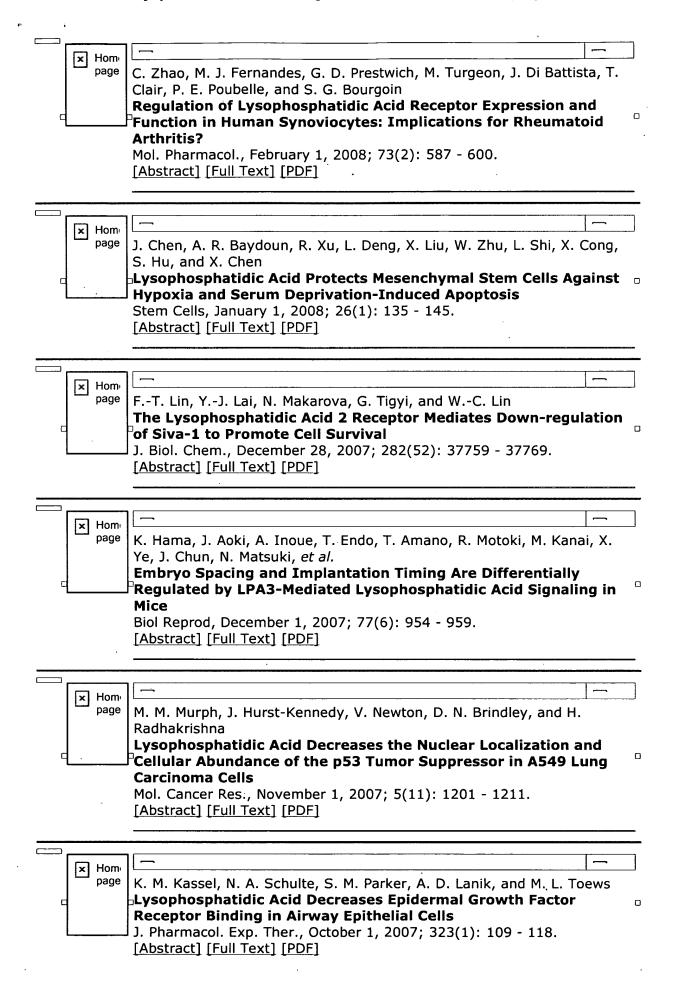
Lysophosphatidic acid (LPA) is a bioactive lipid mediator with diverse physiological and pathological actions on many types of cells. LPA has been widely considered to elicit its biological functions through three types of G protein-coupled receptors, Edg (endothelial cell differentiation gene) 2/LPA1/Vzg (ventricular zone gene) 1, Edg4/LPA2 and Edg7/LPA3. We identified an orphan G protein-coupled receptor, p2y9/GPR23, as the fourth LPA receptor (LPA4). Membrane fractions of RH7777 cells transiently expressing p2y9/GPR23 displayed a specific binding

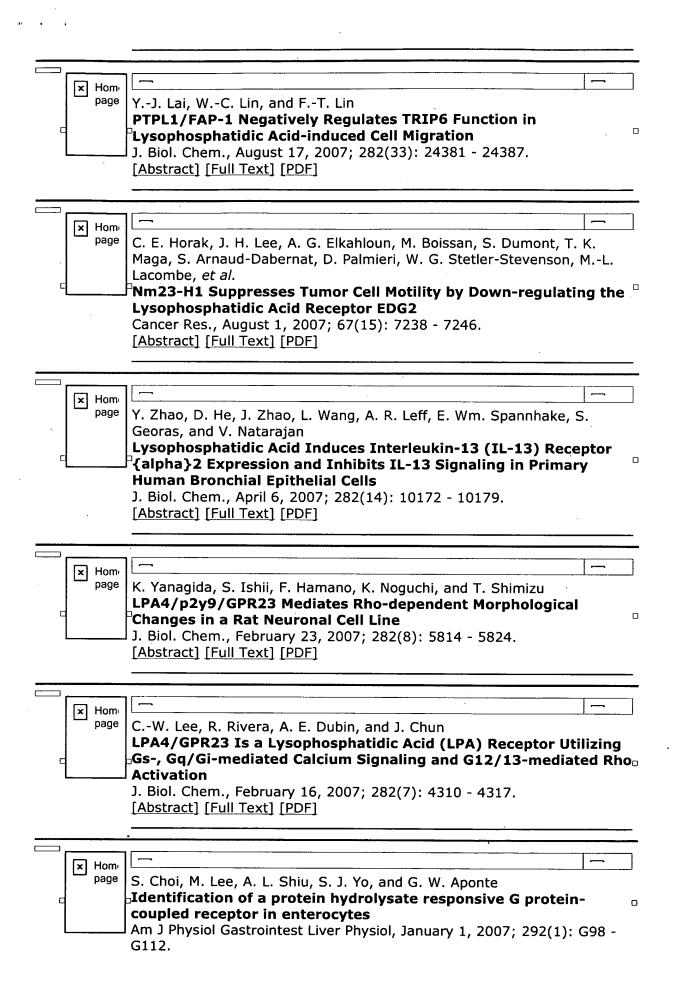
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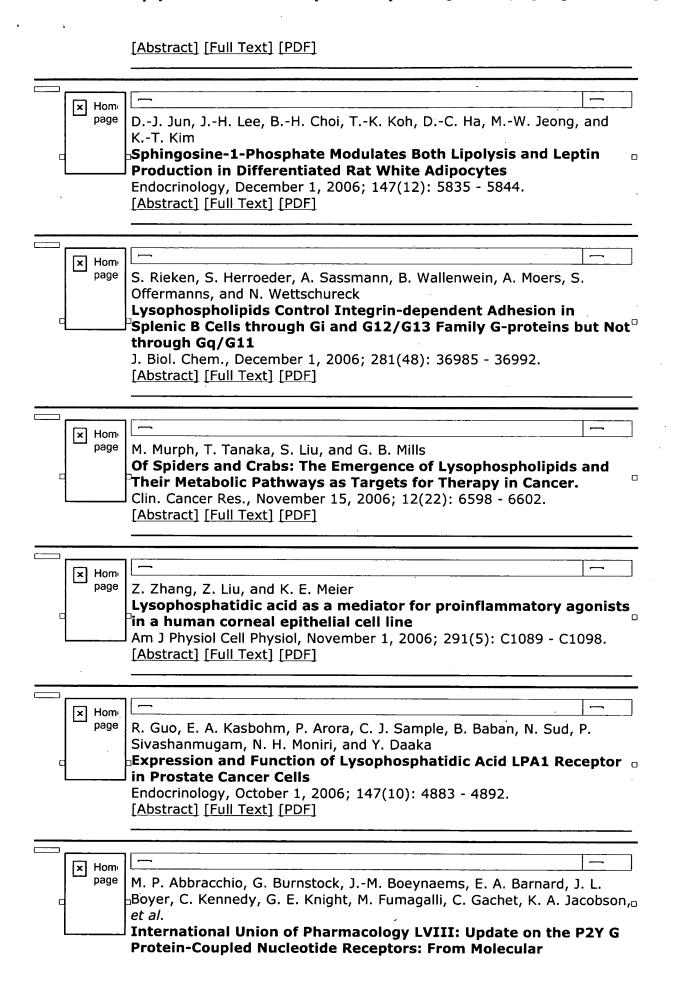
Articles by Shimizu, T.

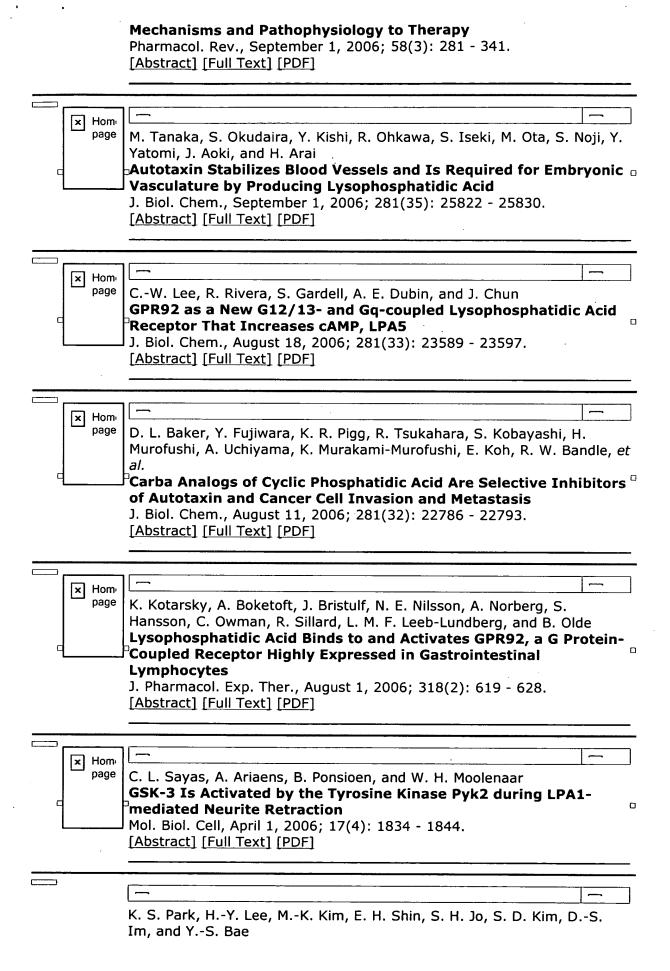
for 1-oleoyl-LPA with a Kd value of around 45 nM. Competition binding and reporter gene assays showed that p2y9/GPR23 preferred structural analogs of LPA with a rank order of 1-oleoyl- > 1-stearoyl- > 1-palmitoyl- > 1-myristoyl- > 1-alkyl- > 1-alkenyl-LPA. In Chinese hamster ovary cells expressing p2y9/GPR23, 1-oleoyl-LPA induced an increase in [Ca2+]i, and stimulated adenylyl cyclase activity. Quantitative Real time-PCR demonstrated that mRNA of p2y9/GPR23 was significantly abundant in ovary compared to other tissues. Interestingly, p2y9/GPR23 shares only 20-24% amino acid identities with Edg2, Edg4 and Edg7, and phylogenetic analysis also shows that p2y9/GPR23 is far distant from the Edg family. These facts suggest that p2y9/GPR23 has evolved from different ancestor sequences from the Edg family.

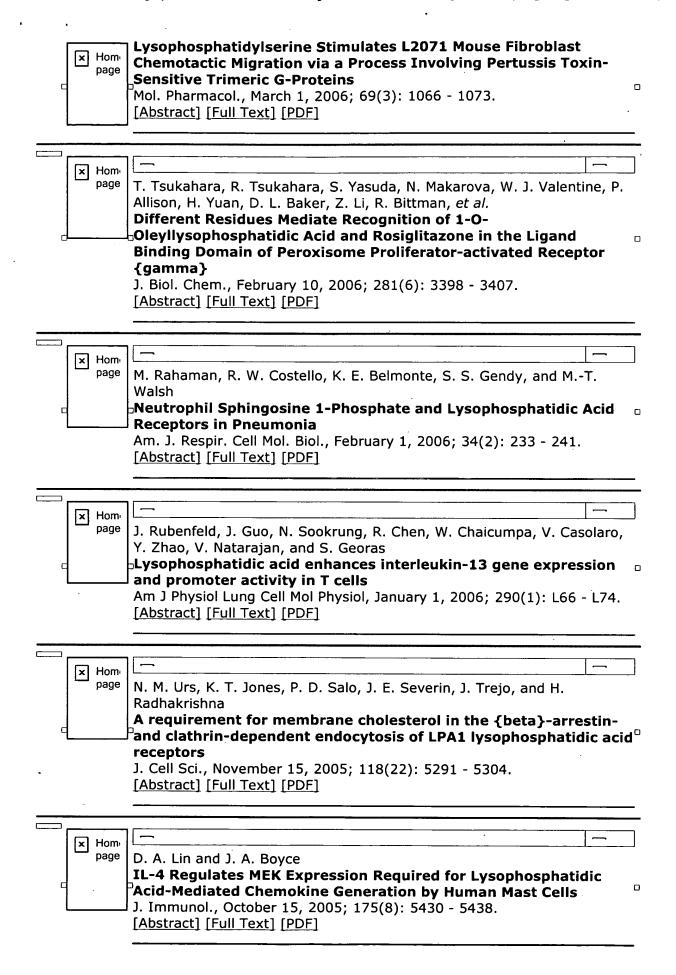
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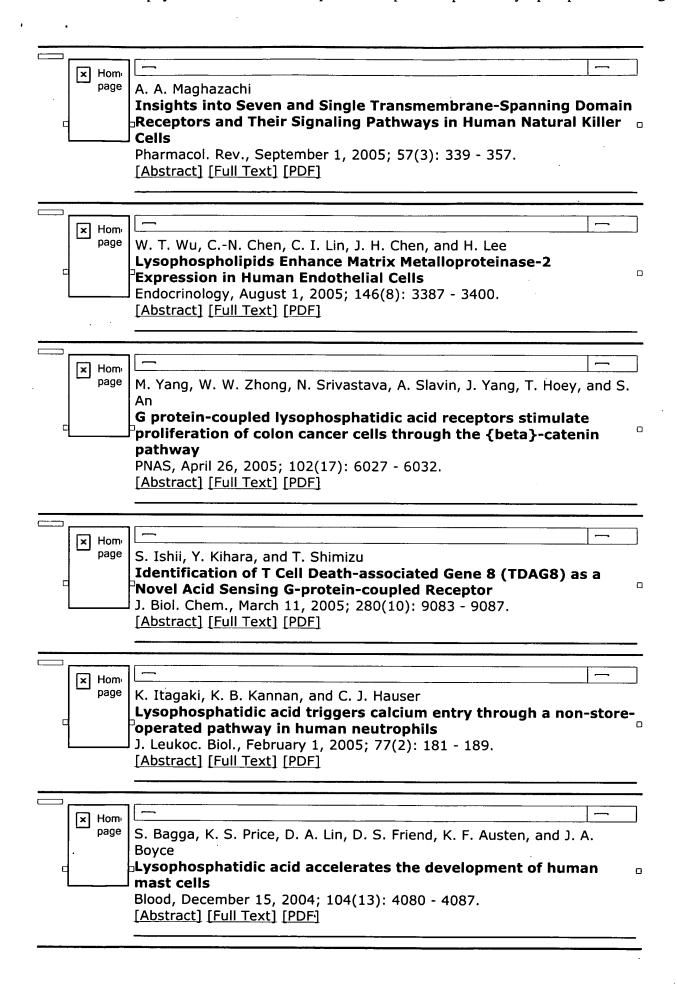


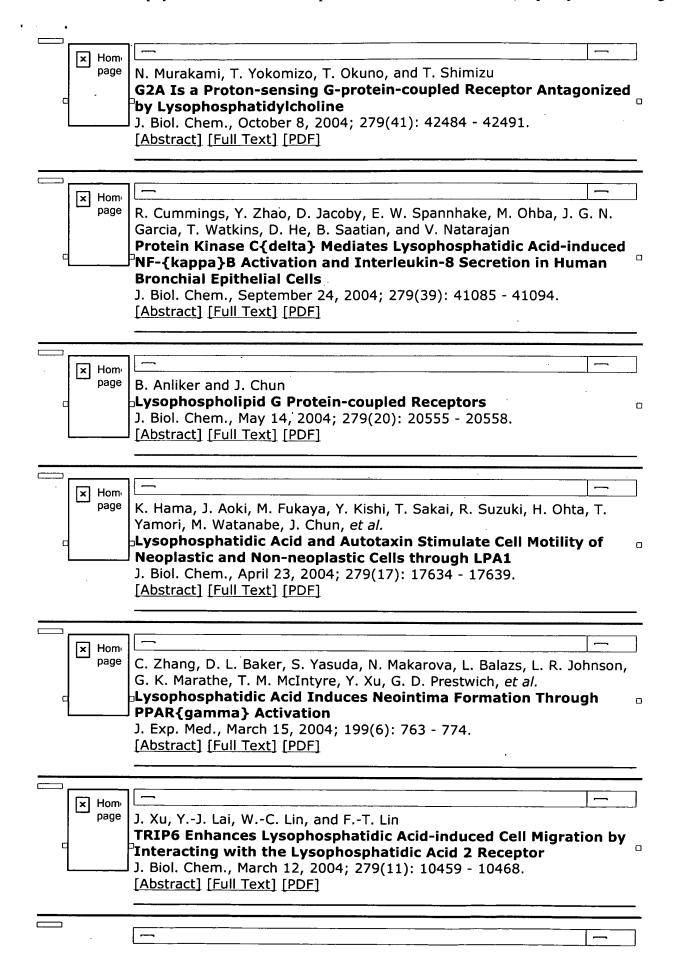


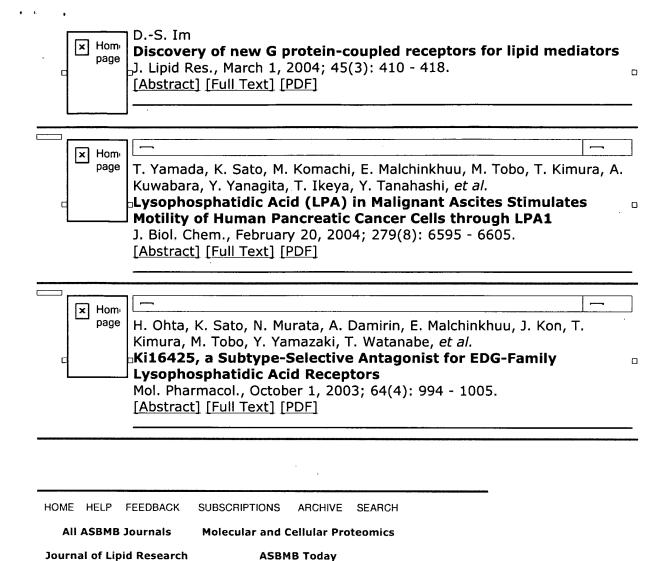












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